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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:

WEEKS et al.

Group Art Unit: 1638

Examiner: D. Kruse

Serial No.: 09/055,145

Filed: April 3, 1998

Atty. File No.: 3553-18

For: "METHODS ANDMATERIALS FOR MAKING AND USING TRANSGENIC DICAMBA-DEGRADING ORGANISMS"

DECLARATION OF DR. DONALD P. WEEKS (Under 37 CFR 1.132)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Dr. Donald P. Weeks, declare that:

1. I am the same Donald P. Weeks who is named as an inventor on the above-referenced patent application.

2. The following discussion and attached data and alignments are presented in support of Applicants' arguments against the rejection of Claims 1, 2, 4, 5, 7, 21, 24, 36, 39, 44, 47, 48, 50-52, 54-56 and 58-65 under 35 U.S.C. § 112, first paragraph. Specifically, the following discussion provides additional evidence that the description in the above-identified specification combined with the knowledge in the art allow one to modify several amino acids in the oxygenase$_{DIC}$ of the present invention (SEQ ID NO:4) while maintaining enzymatic activity of the oxygenase.

3. The *in vivo* assays described below were conducted in my laboratory to further demonstrate that one skilled in the art, given the description provided in the above-identified application and given the knowledge of the structure of oxygenases in

the art, can modify the oxygenase$_{DIC}$ gene of the present invention to cause specific changes in the amino acid sequence of the oxygenase$_{DIC}$ enzyme without causing significant changes in its activity *in vivo* (or causing significant changes, if this is desired). More specifically, these data show that cultures of wild-type *E. coli* transformed with a plasmid that expresses the oxygenase$_{DIC}$ gene of the invention are able to degrade ^{14}C-labeled dicamba to 3,6- dichlorosalicylic acid (DCSA) *in vivo*. Using this assay, we further demonstrate that specific, site-directed mutations in the oxygenase$_{DIC}$ gene can be designed to maintain or affect the level of activity of the oxygenase$_{DIC}$ by simply selecting the amino acid residues to be modified. Finally, we have isolated and sequenced the majority of each of the oxygenase enzymes from the three species of dicamba-degrading bacteria for which Southern blot data was provided in the Declaration under 37 CFR 1.132 filed on July 26, 2001, and we demonstrate that these oxygenases are between 90.1% and 99% identical at the amino acid level to the corresponding portion of the oxygenase$_{DIC}$ represented in the present application by SEQ ID NO:4.

Analysis of Mutations in the Oxygenase$_{DIC}$

First, to demonstrate that one of skill in the art can readily make amino acid changes to the oxygenase$_{DIC}$ of the present invention that do not affect enzyme activity, we chose to mutagenize coding regions for three amino acid residues in the N-terminal region of the oxygenase$_{DIC}$ molecule and two in the C-terminal region (noted by arrows in Figure 1). The amino acid sequence alignments for four separate Rieske non-heme iron-binding oxygenases depicted in Figure 1 demonstrate expected strong homology within the conserved active site domains underlined in the figure and modest, but clear, conservation of amino acid sequence outside these regions. The three oxygenase sequences used in the alignment in Figure 1 were available either prior to the filing of the provisional applications from which this application claims priority (VanA_Ps, 1995 and TxsM, July 1995) or shortly after the filing of the provisional applications (VanA_Ac, June 1997). In addition, the sequences for other Rieske non-heme oxygenase genes were publicly available at the time of the invention (e.g., Brunel and Davidson, J. Bacteriol. 170:4924-4930 (1988)). Therefore, one of skill in the art could have easily performed this or a similar alignment to compare the structure of the oxygenase$_{DIC}$ to known oxygenase structures at the time of the present invention.

I have previously shown, as discussed in the specification and illustrated in the Declaration under 37 CFR 1.132 filed on December 13, 2002, that the amino acid sequence for the oxygenase$_{DIC}$ (SEQ ID NO:4) exhibits two highly conserved consensus sequences that are separately involved in the binding of the Rieske cluster and the "free" iron molecule. We deliberately chose not to mutagenize any of the highly conserved amino acid residues that make up these binding sites for ligands that are essential to the catalysis performed by the enzyme (which incidentally comprise only about 13-15% of the total oxygenase sequence). That is, in evaluating potential modifications to the amino acid sequence of oxygenase$_{DIC}$ (Figure 1, the fourth line labeled DdmC), we deliberately chose not to modify the two cysteine (C) residues and two histidine (H) residues that are essential to the binding of the Rieske [2Fe-2S] cluster within the Rieske [2Fe-2S] domain shown for oxygenase$_{DIC}$ (DdmC) and three other closely related oxygenases (VanA_Ac, VanA-Ps, and TsaM) that, together with oxygenase$_{DIC}$, are members of the Rieske non-heme Fe(II)-binding oxygenase family of enzymes. Neither did we make changes in the two aspartic acid (D) residues or two histidine (H) residues (Figure 1) that are needed for binding of non-heme, "free" iron in most known non-heme free-iron binding domains. Numerous previously published research papers have documented that modification of these residues in an oxygenase almost always leads to dramatic decreases in enzymatic activity and therefore, it is quite predictable that modification of these residues in the dicamba-degrading oxygenase would have a similar effect.

Instead, we chose to produce changes in the amino acid sequence of oxygenase$_{DIC}$ in sites outside these ligand binding sites in order to demonstrate that one of skill in the art can select multiple sites for modification without having a significant detrimental effect on the activity of oxygenase$_{DIC}$. The specific amino acid residues we have changed in the amino acid sequence of oxygenase$_{DIC}$ (Figure 1) are Ala7Gly [i.e., the alanine residue (Ala) at the seventh position from the N-terminus of oxygenase$_{DIC}$ was exchanged for a glycine (Gly) residue], Glu15Asp, Gly23Ala, Ile330Leu and Leu333Val.

a) *Introduction to Experiments*

The oxygenase$_{DIC}$ gene product (i.e., oxygenase$_{DIC}$, represented by SEQ ID NO:4 and shown in Figure 1) has been previously shown by my laboratory to catalyze the conversion of dicamba to 3,6-dichlorosalicylic acid in *Pseudomonas*

maltophilia, strain DI-6. Because *E. coli* contains a ferredoxin enzyme that can substitute for the ferredoxin$_{DIC}$ and reductase$_{DIC}$ from strain DI-6 in allowing the conversion of dicamba to 3,6-DCSA, placement of the oxygenase$_{DIC}$ gene (comprising SEQ ID NO:3) into *E. coli* has allowed for the use of a controlled *in vivo* assay for the activity of wild-type and mutant versions of oxygenase$_{DIC}$. This *in vivo* assay was developed to detect changes (or not) in dicamba O-demethylase activity among mutant enzymes resulting from nucleotide modifications in the dicamba O-demethylase oxygenase$_{DIC}$ gene. Comparisons of the percentage of dicamba degradation among bacteria containing plasmids expressing mutant oxygenase proteins were used to establish the requirement for specific amino acids in supporting the enzymatic activity of oxygenase$_{DIC}$.

b) *Materials and Methods*

Mutagenesis : The Quickchange Mutagenesis kit (Stratagene) was used to make several mutations in the oxygenase gene. The wild type oxygenase$_{DIC}$ gene in an *E. coli* pET expression vector was used as the template. A set of complementary primers were designed for each mutation. PCR directed mutation reactions were done according to manufacturer's recommendations. The parental DNA strand was removed by digestion with DpnI for 1 hour at 37°C. The PCR product containing the mutation was used to transform BL21 cells. Mutations were confirmed by DNA sequencing. The mutant plasmids used for *in vivo* experiments were designated pA7G, pE15D, pG23A, pI330L and pL333V.

The primers used for generating the mutants are listed below:

PA7G: 5'-CCT TCG TCC GCA ATG **G**CT GGT ATG TGG C-3'
5'-GCC ACA TAC CAG **C**CA TTG CGG ACG AAG G-3'

pE15D: 5'-GGC GCT GCC CGA **C**GA ACT GTC CGA AAA GC-3'
5'-GCT TTT CGG ACA GTT C**G**T CGG GCA GCG CC-3'

pG23A: 5'-CGA AAA GCC GCT CG**C** CCG GAC GAT TCT CG-3'
5'-CGA GAA TCG TCC GG**G** CGA GCG GCT TTT CG-3'

pI330L: 5'-CGT GTC AGC CGC GAG **C**TC GAG AAG CTT GAG C-3'
5'-GCT CAA GCT TCT CGA **G**CT CGC GGC TGA CAC G-3'

pL333V: 5'-CGA GAT CGA GAA G**G**T TGA GCA GCT CG-3'

5'-CGA GCT GCT CAA **CCT** TCT CGA TCT CG-3'

The nucleotides in **boldface** represent the mutation inserted into the oxygenase$_{DIC}$ gene.

***In vivo* radioactive assay for DCSA:** *E. coli* (strain BL21) cells transformed with an expression vector containing the wild-type oxygenase$_{DIC}$ gene or one of the five mutagenized versions of the oxygenase$_{DIC}$ gene (described above) were grown in separate Petri dishes containing solid LB/Amp medium in order to produce individual colonies. Inoculation loop picks from single colonies were used to inoculate 37°C overnight cultures of *E. coli* (5 ml of LB/Amp). BL21 cells lacking a plasmid were treated identically and used as negative controls to demonstrate that wild-type *E. coli* cells are not capable of converting dicamba to 3,6-DCSA. An aliquot of the overnight culture was used to inoculate 4 ml of LB/Amp medium. The cultures were incubated at 25°C to an O.D. between 0.4-0.6 at which time they were induced with IPTG at a final concentration of 1mM. Incubation was continued overnight at a lower temperature of 15°C. After the overnight incubation, cultures (1.5 ml) were exposed to ^{14}C-labeled dicamba at a final concentration of 50 μM for an additional 18-24 hrs at 15°C. Enzymatic conversion of dicamba to DCSA was terminated by addition of 240ul of 5% sulfuric acid. The cell suspension was centrifuged to pellet cells and the supernatant was extracted twice with an equal volume of ether. To maintain controlled experimental conditions, the ether extracts were evaporated to dryness. A known volume (50 μl) of ether was then added to each sample, vortexed thoroughly and spotted on aluminum backed UV/silica TLC plates. An *in vitro* assay using strain DI-6 lysate was performed essentially as described (Wang et al., 1997). The reaction product was run on the same TLC plate and served as a positive identification for DCSA migration. The TLC plates were developed using a mobile phase composed of chloroform:ethanol:acetic acid in the ratio of 85:10:5. The dried TLC plates were then exposed to a phosphorimager screen. Images of the radioactivity detected by the phosphorimager screen were analyzed using the 'Quantity One' software. Each experiment was performed in duplicate in an attempt to establish reproducibility.

c) *Results*

E. coli containing the oxygenase$_{DIC}$ gene under the control of beta-galactosidase inducible promoter were able to convert ^{14}C-dicamba to 3,6-DCSA during an overnight incubation in the presence of the inducer, ITPG [Figure 2, lane 7 (WT Oxy Gene)]. *E. coli* lacking this gene demonstrated no ability to degrade dicamba to DCSA [Figure 2, lane 8 (No Oxy Gene)] under identical conditions. When *E. coli* is transformed with an expression vector carrying an oxygenase$_{DIC}$ gene that has been subjected to site-directed mutagenesis, the amount of enzymatic activity detected depends on which amino acid in the oxygenase$_{DIC}$ has been changed. For example, changing alanine residue in position seven to a glycine residue (i.e., Ala7Gly; lane 1, Figure 2) results in slight, if any, decrease in enzymatic activity. Likewise, conversion of the glycine at position 23 to an alanine (Gly23Ala; lane 3, Figure 2) provides as much, if not slightly more, enzymatic activity as observed in *E. Coli* cells bearing the wild-type form of oxygenase$_{DIC}$. Conversion of the glutamic acid residue at position 15 to an aspartic acid residue resulted in nearly complete loss of enzymatic activity (Glu15Asp; lane 2, Figure 2). Two other modifications (i.e., Ile330Leu; lane 4 and Leu334Ile; lane 5) resulted in enzymes with intermediate levels of activity. The results of the experiments described above (Experiment #1) were shown to be reproducible in a second independent experiment (Experiment #2) performed with new cultures of *E. coli* carrying the same plasmid constructs (Table 1). Cultures used in the second experiment were at a slightly different stage of growth and produced less enzymatic activity during each incubation; nonetheless, the results are qualitatively the same.

d) *Discussion*

Results from experiments presented here provide strong evidence that the oxygenase$_{DIC}$ gene of the present invention can be readily mutated to produce changes in the amino acid sequence of oxygenase$_{DIC}$ that do not eliminate the enzymatic activity of the enzyme in cultures of *E. coli* expressing these mutated genes, since 4 out of the 5 modifications selected resulted in functional enzymes (e.g., Ala7Gly, Gly23Ala, Ile330Leu and Leu334Ile; Figure 2 and Table 1).

Importantly, these enzymes are all useful for the production of dicamba-resistant transgenic plants, because we have previously demonstrated that transgenic plants expressing even *low levels* of oxygenase activity are resistant to treatment with *moderate to high levels* of dicamba (data not shown), and therefore, even mutated genes conferring *intermediate* levels of oxygenase$_{DIC}$ activity (e.g., Ile330Leu and Leu334Ile) can provide adequate protection to transgenic plants carrying these genes.

Moreover, it is noted that the five selected mutations were at positions where the amino acids are relatively well conserved among oxygenases. One can readily see from the amino acid alignment in Figure 1 that there are numerous additional positions that are relatively non-conserved between the dicamba-degrading oxygenase and the other exemplified oxygenases, and it is well known in the art that such positions are even better targets for modification as they can be predicted to be less likely to disrupt enzyme function. Referring to Figure 1 again, there are at least 114 non-conserved amino acid residues in the regions between positions 1-40 and positions 166-339 of SEQ ID NO:4, which are suitable targets for modification and which comprise about 33.6% of the protein, not even taking into account the possibility of modifying moderately conserved, "similar" residues, or the fact that the five modifications described in the experiments described above (80% of which produced functional enzymes) were all in *conserved* positions of the protein. The point of these experiments, the alignment, and the discussion of what was already known about the active site of an oxygenase, is that it should be clear that one of skill in the art has sufficient information based on the specification and the level of knowledge in the art regarding oxygenases to predictably be able to change up to 35% of the oxygenase sequence while maintaining enzyme function. Indeed, I have previously argued (referring to the December 13 Declaration) that, based on studies of other enzymes, amino acid substitutions can be observed in nature in nearly one-half (**50%**) of the positions of within the amino acid sequence while maintaining the enzymatic activity of the enzyme. The data provided herein clearly support my prior position.

Given that mutations in amino acid sequence in four out of five positions in oxygenase$_{DIC}$ maintained sufficient enzyme activity to predictably protect a transgenic plant from dicamba exposure, it can be predicted that changes in up to at least 35% of the positions in the oxygenase$_{DIC}$ amino acid sequence would yield enzymes with sufficient activity to produce dicamba-resistant transgenic plants when the gene encoding oxygenase$_{DIC}$ is appropriately modified with promoter and termination regions allowing for expression of oxygenase$_{DIC}$ in higher plants.

Sequence Analysis of Other Dicamba-Degrading Bacterial Species

To further emphasize that one can make predictions about the structure-to-function relationship of a previously unknown protein based on comparison to proteins of similar function, and to further emphasize that one of skill in the art will expect other dicamba-degrading oxygenases to have structures similar to that of SEQ ID NO:4, I make the following observations. The availability of the tertiary structure naphthalene dioxygenase (known from detailed X-ray crystallographic analyses; Karlsson A, Parales JV, Parales RE, Gibson DT, Eklund H, Ramaswamy S. Crystal structure of naphthalene dioxygenase: side-on binding of dioxygen to iron. Science 2003 299:1039-42) allows us to illustrate by comparison with a tertiary structure of oxygenase$_{DIC}$ (Figure 3), that there are dramatic similarities in the overall conformation of the enzyme subunits as well as strong conservation of placement of the active site ligands (small red balls at the upper left of the subunits represents placement of the "free" iron moiety and the yellow and red balls at the bottom of the molecule represents the Rieske [2Fe-2S] cluster). These analyses leave no doubt that oxygenase$_{DIC}$ is a typical member of the Rieske non-heme iron-binding family of oxygenases, even though they differ in primary amino acid sequences and substrate specificity. From this realization and the realization that a multitude of different dicamba-degrading microorganisms with dicamba degrading enzymes are found in large numbers in virtually all agronomic soils throughout the world, it is logical to believe that in Nature, one would expect to find dicamba-degrading Rieske non-heme iron-binding oxygenases that fall within the scope of the present claims.

To demonstrate this point, we have previously provided data regarding three different species of dicamba-degrading bacteria (designated as strains BRW1, BRW2 and BRW3) isolated from soil at the same dicamba-manufacturing site as was the *Pseudomonas maltophilia*, strain DI-6 (the source of the gene encoding oxygenase$_{DIC}$) (see July 21, 2001 Declaration under 37 CFR 1.132). Two of these strains are of a different species than the *Pseudomonas maltophilia*, strain DI-6 (*Pseudomonas* sp. BRW 1, *Pseudomonas* sp. BRW 3), and one is of a different genus (*Sphingomonas* sp. BRW 2). Estimates from DNA hybridization experiments in which the stringency of hybridization conditions was varied indicated that the oxygenase gene from strain DI-6 was probably at least 90% identical in DNA sequence to the oxygenase genes in strains BRW1, BRW2 and BRW3 (See July 21 Declaration). Furthermore, we have demonstrated that the first step in degradation of dicamba by these bacteria is the oxidation of dicamba to produce 3,6-dichlorosalicyic acid, thus demonstrating that these bacteria contain a dicamba-degrading oxygenase with the same activity as the oxygenase$_{DIC}$ represented by SEQ ID NO:4.

Recent DNA sequencing of the majority of the coding regions of oxygenase genes from each of these three separate bacterial strains (BRW1, BRW2, and BRW3) have demonstrated that the oxygenase genes from two out of the three bacteria (BRW1 and BRW3) are nearly identical to the comparable region of the coding region of the oxygenasse$_{DIC}$ gene from strain DI-6 (Table 2; 99.9% and 99.9% identical, respectively). The oxygenase gene from BRW2 is about 98.1% identical to the oxygenase$_{DIC}$ gene from strain DI-6 over the majority of the coding sequence. When translated into amino acid sequence, we find that there is 99% identity between oxygenases from BRW 1 and BRW3 compared with the comparable portion of the oxygenase$_{DIC}$ from Strain DI-6 (positions 15-324 of SEQ ID NO:4), and the oxygenase from BRW2 is 90.1% identical to oxygenase$_{DIC}$ from strain DI-6 (over positions 15-326 of SEQ ID NO:4) (See attached Alignments). Due to restrictions based on the primer design for this initial sequencing (see below), the identity over the N- and C-terminal regions of the BRW1, BRW2 and BRW3 oxygenases is not available. More specifically, for

these experiments, we prepared polymerase chain reaction (PCR) primers that were designed to hybridize to the extreme 5' and 3' regions of the oxygenase gene coding region. Thus, the DNA sequence information and amino acid sequence information presented here do not cover the first 14 codons or the last 13 codons of the oxygenase gene coding sequence for BRW1, BRW2 and BRW3. However, based on the initial sequencing, and the information provided by the alignment of the oxygenasse$_{DIC}$ with other non-dicamba-degrading oxygenases, one of skill in the art will expect that the N- and C-terminal regions will have a similar level of identity to the comparable regions of SEQ ID NO:4.

These data rigorously demonstrate that Rieske non-heme iron-binding oxygenases from naturally-occurring, dicamba-degrading bacterial species other than *Pseudomonas maltophilia*, strain DI-6 can convert dicamba to DCSA and produce oxygenases with amino acid sequences that fall within the scope of the present claims.

Further research in our laboratory has shown that the oxygenase in all four of the bacterial species and strains (DI-6, BRW1, BRW2, and BRW3) are located on large plasmids that likely spread horizontally (i.e., by genetic exchange) through bacterial communities in which dicamba (or another unknown substrate for oxygenase$_{DIC}$) is sufficiently abundant to provide a useful source of carbon and energy for bacterial growth. Again, this finding emphasizes that in nature there likely exist many dicamba-degrading Rieske non-heme iron-binding oxygenases that will fall within the scope of the present claims.

4. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: March 5, 2001 By: Donald P. Weeks

Donald P. Weeks

Figure 1. Amino Acid Sequences of Four Oxygenases

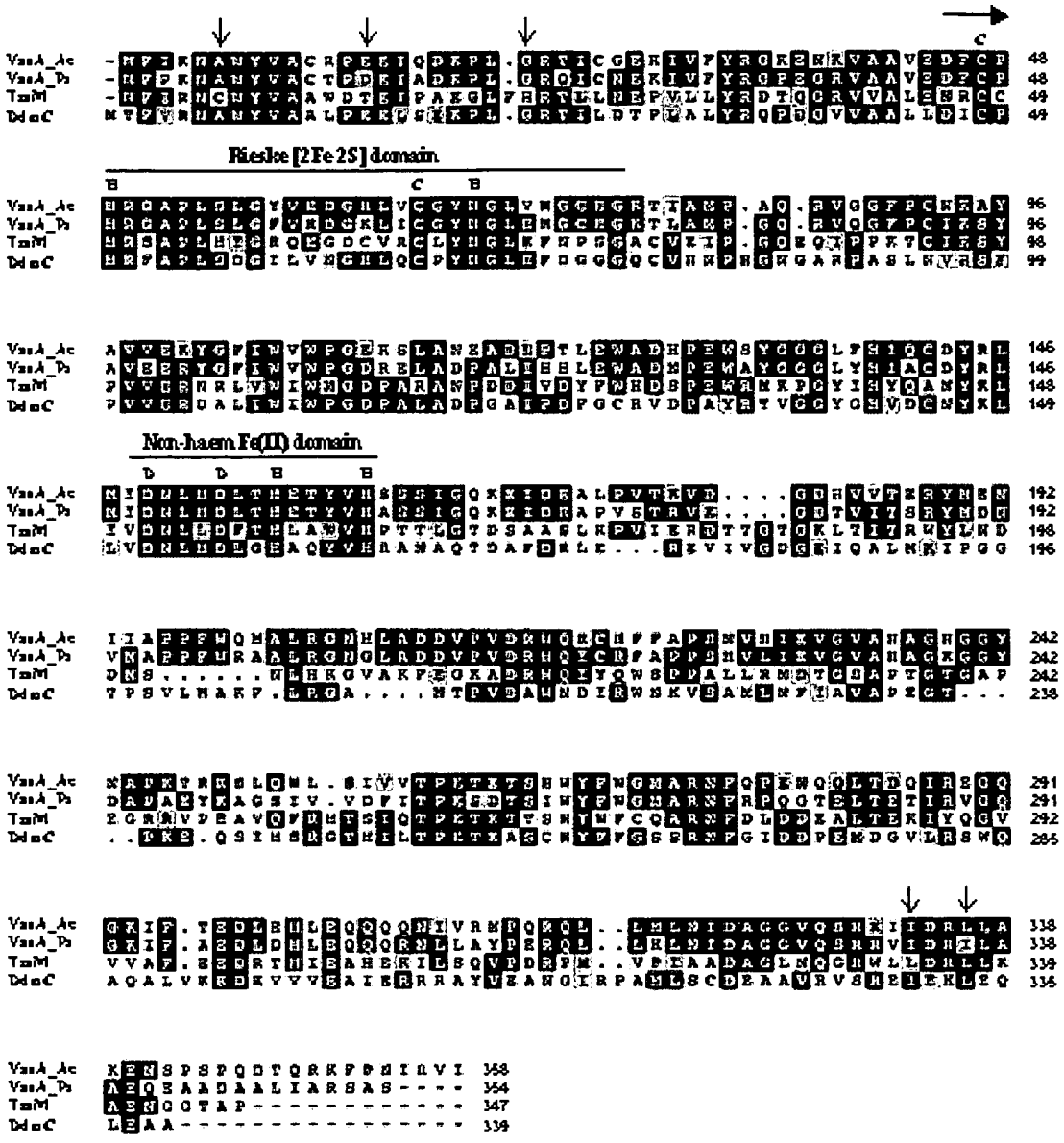


Figure 2. Dicamba Degradation by Wild-type and Mutant Oxygenase$_{DIC}$ Enzymes in *E. coli*

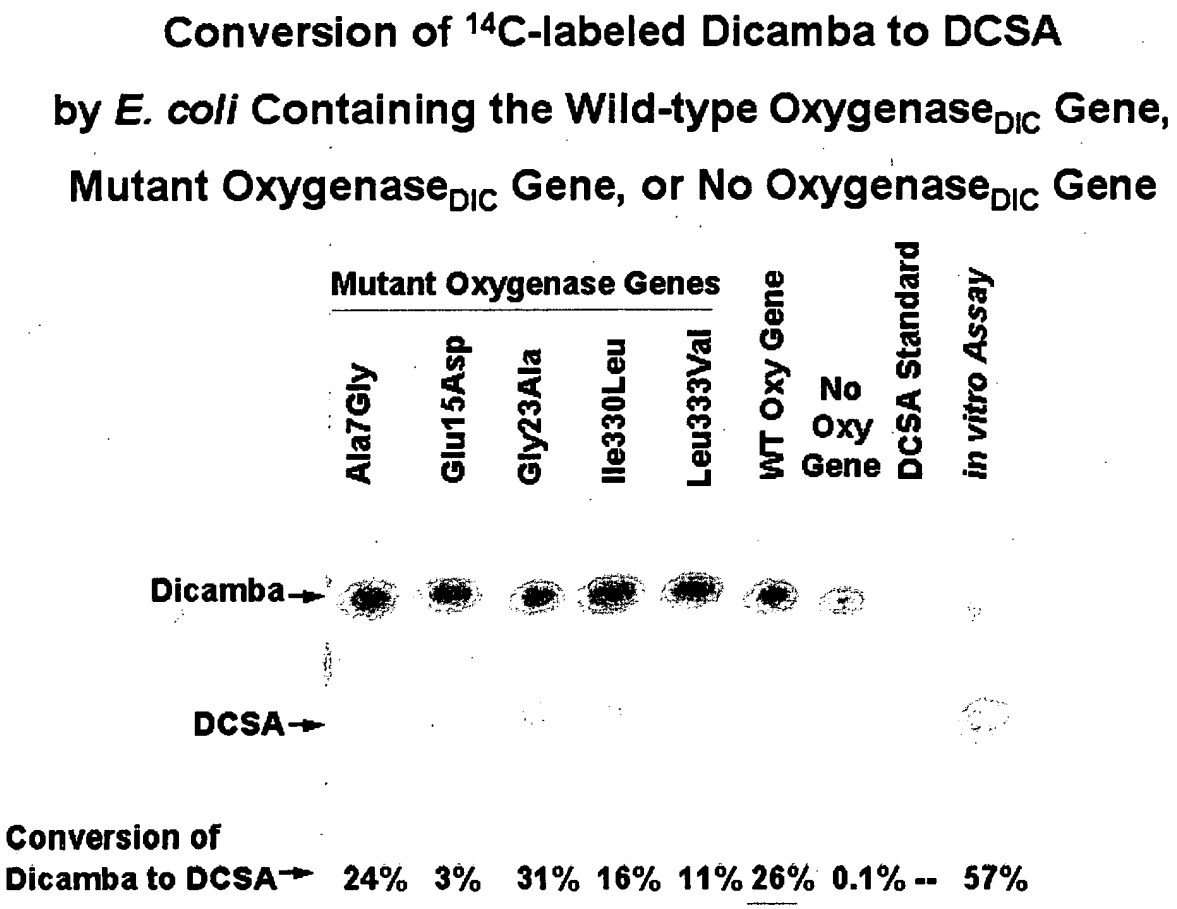
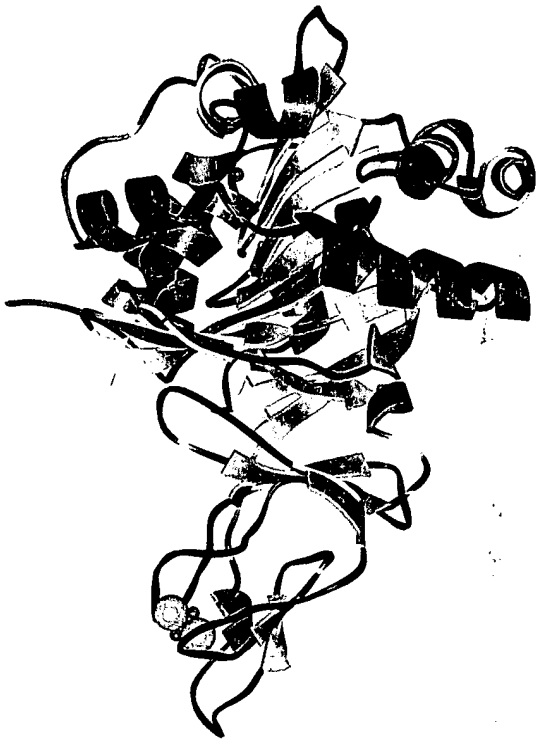


Figure 3. Comparison of 3-D Structures

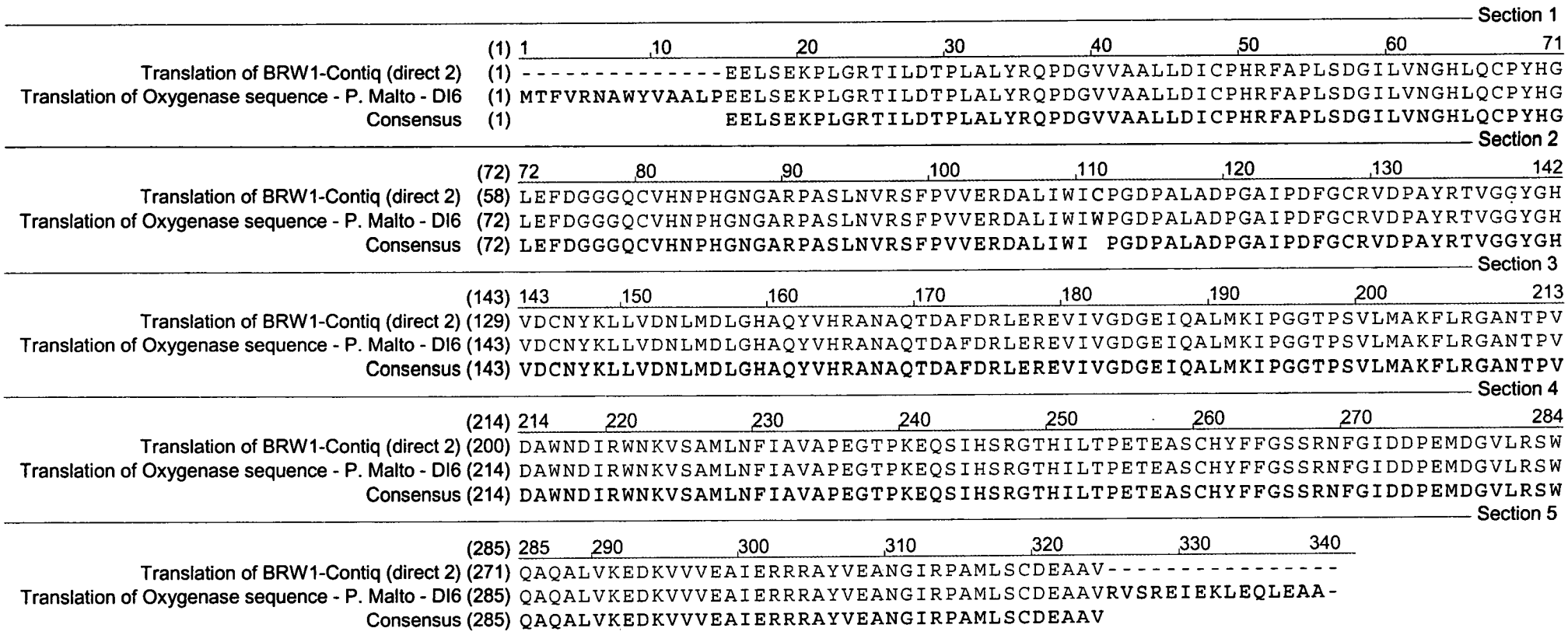
Naphthalene Dioxygenase Subunit



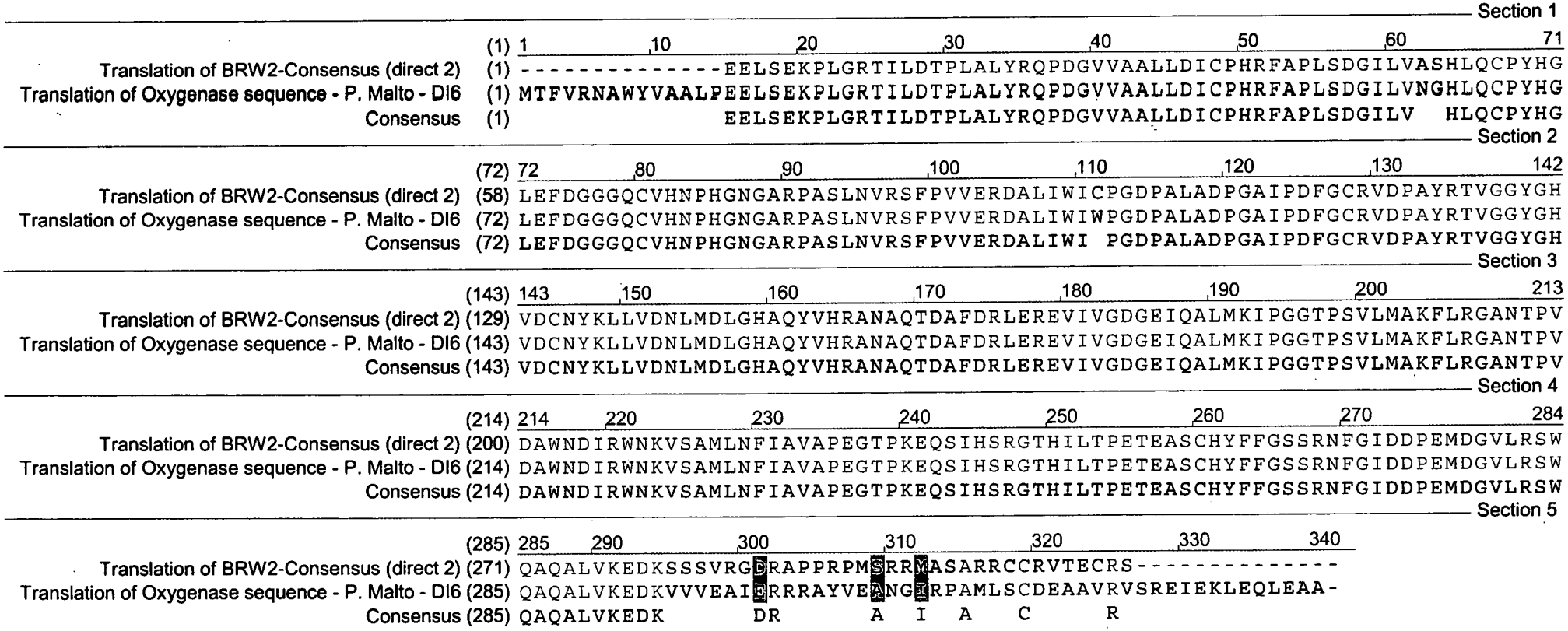
Oxygenase$_{DIC}$ Subunit

Table 1. Conversion of Dicamba to DCSA *in vivo* by *E. coli* Cultures Carrying either a Wild-type Oxygenase$_{DIC}$ Gene or Mutant Oxygenase$_{DIC}$ Genes Modified *in vitro* by Site-directed Mutagenesis

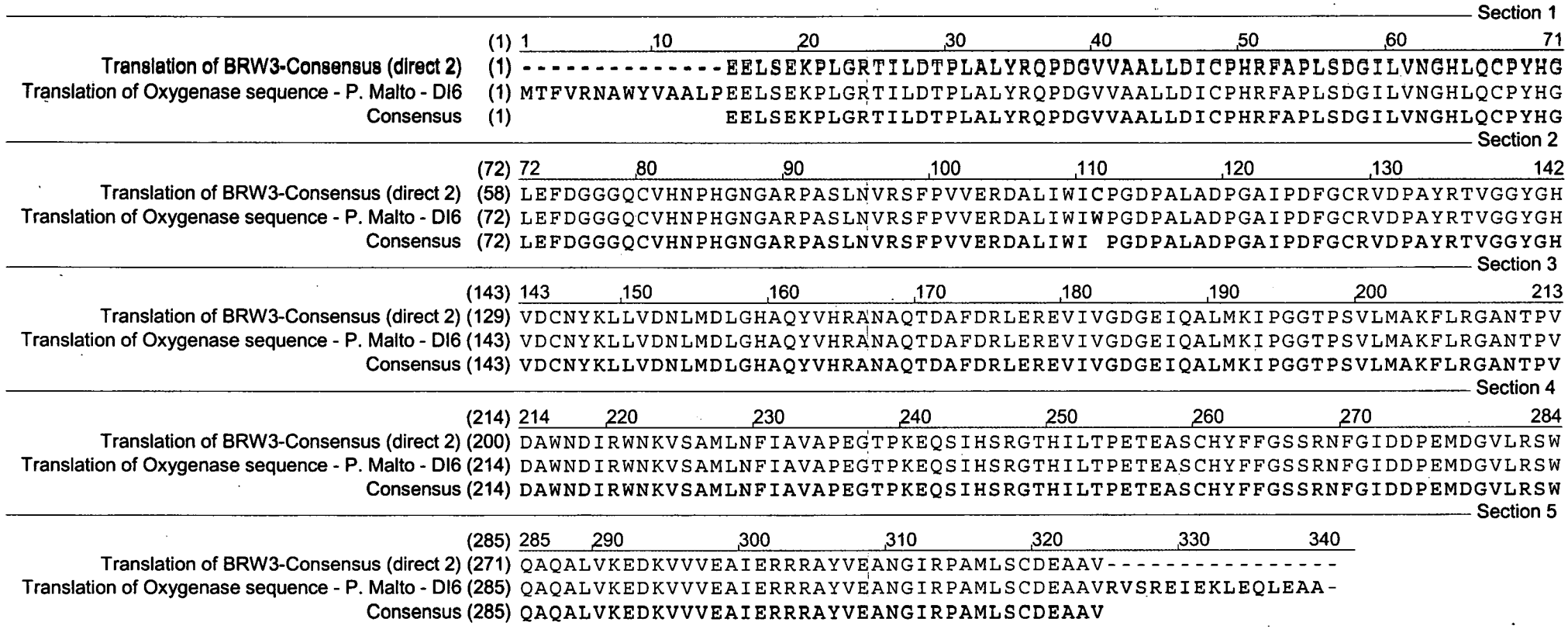
	Conversion Of Dicamba to DCSA (%)	
Sample	Expt. #1	Expt. #2
No Oxygenase gene	0.1	0.4
Wild-Type Oxygenase gene	26	18
Ala7Gly Mutant Oxygenase gene	24	12
Glu15Asp Mutant Oxygenase gene	3	0.6
Gly23Ala Mutant Oxygenase gene	31	14
Ile330Leu Mutant Oxygenase gene	16	6.5
Leu333Val Mutant Oxygenase gene	11	4
In vitro Assay	57	20


Section 1
(1) 1 10 20 30 40 50 60 71
Translation of BRW1-Contiq (direct 2) (1) ----------EELSEKPLGRTILDTPLALYRQPDGVVAALLDICPHRFAPLSDGILVNGHLQCPYHG
Translation of Oxygenase sequence - P. Malto - DI6 (1) MTFVRNAWYVAALPEELSEKPLGRTILDTPLALYRQPDGVVAALLDICPHRFAPLSDGILVNGHLQCPYHG
Consensus (1) EELSEKPLGRTILDTPLALYRQPDGVVAALLDICPHRFAPLSDGILVNGHLQCPYHG
Section 2
(72) 72 80 90 100 110 120 130 142
Translation of BRW1-Contiq (direct 2) (58) LEFDGGGQCVHNPHGNGARPASLNVRSFPVVERDALIWICPGDPALADPGAIPDFGCRVDPAYRTVGGYGH
Translation of Oxygenase sequence - P. Malto - DI6 (72) LEFDGGGQCVHNPHGNGARPASLNVRSFPVVERDALIWIWPGDPALADPGAIPDFGCRVDPAYRTVGGYGH
Consensus (72) LEFDGGGQCVHNPHGNGARPASLNVRSFPVVERDALIWI PGDPALADPGAIPDFGCRVDPAYRTVGGYGH
Section 3
(143) 143 150 160 170 180 190 200 213
Translation of BRW1-Contiq (direct 2) (129) VDCNYKLLVDNLMDLGHAQYVHRANAQTDAFDRLEREVIVGDGEIQALMKIPGGTPSVLMAKFLRGANTPV
Translation of Oxygenase sequence - P. Malto - DI6 (143) VDCNYKLLVDNLMDLGHAQYVHRANAQTDAFDRLEREVIVGDGEIQALMKIPGGTPSVLMAKFLRGANTPV
Consensus (143) VDCNYKLLVDNLMDLGHAQYVHRANAQTDAFDRLEREVIVGDGEIQALMKIPGGTPSVLMAKFLRGANTPV
Section 4
(214) 214 220 230 240 250 260 270 284
Translation of BRW1-Contiq (direct 2) (200) DAWNDIRWNKVSAMLNFIAVAPEGTPKEQSIHSRGTHILTPETEASCHYFFGSSRNFGIDDPEMDGVLRSW
Translation of Oxygenase sequence - P. Malto - DI6 (214) DAWNDIRWNKVSAMLNFIAVAPEGTPKEQSIHSRGTHILTPETEASCHYFFGSSRNFGIDDPEMDGVLRSW
Consensus (214) DAWNDIRWNKVSAMLNFIAVAPEGTPKEQSIHSRGTHILTPETEASCHYFFGSSRNFGIDDPEMDGVLRSW
Section 5
(285) 285 290 300 310 320 330 340
Translation of BRW1-Contiq (direct 2) (271) QAQALVKEDKVVVEAIERRRAYVEANGIRPAMLSCDEAAV----------
Translation of Oxygenase sequence - P. Malto - DI6 (285) QAQALVKEDKVVVEAIERRRAYVEANGIRPAMLSCDEAAVRVSREIEKLEQLEAA-
Consensus (285) QAQALVKEDKVVVEAIERRRAYVEANGIRPAMLSCDEAAV

Alignment 1


Section 1
(1) 1 10 20 30 40 50 60 71
Translation of BRW2-Consensus (direct 2) (1) ----------EELSEKPLGRTILDTPLALYRQPDGVVAALLDICPHRFAPLSDGILVASHLQCPYHG
Translation of Oxygenase sequence - P. Malto - DI6 (1) MTFVRNAWYVAALPEELSEKPLGRTILDTPLALYRQPDGVVAALLDICPHRFAPLSDGILVNGHLQCPYHG
Consensus (1) EELSEKPLGRTILDTPLALYRQPDGVVAALLDICPHRFAPLSDGILV HLQCPYHG
Section 2
(72) 72 80 90 100 110 120 130 142
Translation of BRW2-Consensus (direct 2) (58) LEFDGGGQCVHNPHGNGARPASLNVRSFPVVERDALIWICPGDPALADPGAIPDFGCRVDPAYRTVGGYGH
Translation of Oxygenase sequence - P. Malto - DI6 (72) LEFDGGGQCVHNPHGNGARPASLNVRSFPVVERDALIWIWPGDPALADPGAIPDFGCRVDPAYRTVGGYGH
Consensus (72) LEFDGGGQCVHNPHGNGARPASLNVRSFPVVERDALIWI PGDPALADPGAIPDFGCRVDPAYRTVGGYGH
Section 3
(143) 143 150 160 170 180 190 200 213
Translation of BRW2-Consensus (direct 2) (129) VDCNYKLLVDNLMDLGHAQYVHRANAQTDAFDRLEREVIVGDGEIQALMKIPGGTPSVLMAKFLRGANTPV
Translation of Oxygenase sequence - P. Malto - DI6 (143) VDCNYKLLVDNLMDLGHAQYVHRANAQTDAFDRLEREVIVGDGEIQALMKIPGGTPSVLMAKFLRGANTPV
Consensus (143) VDCNYKLLVDNLMDLGHAQYVHRANAQTDAFDRLEREVIVGDGEIQALMKIPGGTPSVLMAKFLRGANTPV
Section 4
(214) 214 220 230 240 250 260 270 284
Translation of BRW2-Consensus (direct 2) (200) DAWNDIRWNKVSAMLNFIAVAPEGTPKEQSIHSRGTHILTPETEASCHYFFGSSRNFGIDDPEMDGVLRSW
Translation of Oxygenase sequence - P. Malto - DI6 (214) DAWNDIRWNKVSAMLNFIAVAPEGTPKEQSIHSRGTHILTPETEASCHYFFGSSRNFGIDDPEMDGVLRSW
Consensus (214) DAWNDIRWNKVSAMLNFIAVAPEGTPKEQSIHSRGTHILTPETEASCHYFFGSSRNFGIDDPEMDGVLRSW
Section 5
(285) 285 290 300 310 320 330 340
Translation of BRW2-Consensus (direct 2) (271) QAQALVKEDKSSSVRGDRAPPRPMSRRMASARRCCRVTECRS----------
Translation of Oxygenase sequence - P. Malto - DI6 (285) QAQALVKEDKVVVEAIERRRAYVEANGLRPAMLSCDEAAVRVSREIEKLEQLEAA-
Consensus (285) QAQALVKEDK DR A I A C R

Alignment 2


Section 1
(1) 1 10 20 30 40 50 60 71
Translation of BRW3-Consensus (direct 2) (1) ----------EELSEKPLGRTILDTPLALYRQPDGVVAALLDICPHRFAPLSDGILVNGHLQCPYHG
Translation of Oxygenase sequence - P. Malto - DI6 (1) MTFVRNAWYVAALPEELSEKPLGRTILDTPLALYRQPDGVVAALLDICPHRFAPLSDGILVNGHLQCPYHG
Consensus (1) EELSEKPLGRTILDTPLALYRQPDGVVAALLDICPHRFAPLSDGILVNGHLQCPYHG
Section 2
(72) 72 80 90 100 110 120 130 142
Translation of BRW3-Consensus (direct 2) (58) LEFDGGGQCVHNPHGNGARPASLNVRSFPVVERDALIWICPGDPALADPGAIPDFGCRVDPAYRTVGGYGH
Translation of Oxygenase sequence - P. Malto - DI6 (72) LEFDGGGQCVHNPHGNGARPASLNVRSFPVVERDALIWIWPGDPALADPGAIPDFGCRVDPAYRTVGGYGH
Consensus (72) LEFDGGGQCVHNPHGNGARPASLNVRSFPVVERDALIWI PGDPALADPGAIPDFGCRVDPAYRTVGGYGH
Section 3
(143) 143 150 160 170 180 190 200 213
Translation of BRW3-Consensus (direct 2) (129) VDCNYKLLVDNLMDLGHAQYVHRANAQTDAFDRLEREVIVGDGEIQALMKIPGGTPSVLMAKFLRGANTPV
Translation of Oxygenase sequence - P. Malto - DI6 (143) VDCNYKLLVDNLMDLGHAQYVHRANAQTDAFDRLEREVIVGDGEIQALMKIPGGTPSVLMAKFLRGANTPV
Consensus (143) VDCNYKLLVDNLMDLGHAQYVHRANAQTDAFDRLEREVIVGDGEIQALMKIPGGTPSVLMAKFLRGANTPV
Section 4
(214) 214 220 230 240 250 260 270 284
Translation of BRW3-Consensus (direct 2) (200) DAWNDIRWNKVSAMLNFIAVAPEGTPKEQSIHSRGTHILTPETEASCHYFFGSSRNFGIDDPEMDGVLRSW
Translation of Oxygenase sequence - P. Malto - DI6 (214) DAWNDIRWNKVSAMLNFIAVAPEGTPKEQSIHSRGTHILTPETEASCHYFFGSSRNFGIDDPEMDGVLRSW
Consensus (214) DAWNDIRWNKVSAMLNFIAVAPEGTPKEQSIHSRGTHILTPETEASCHYFFGSSRNFGIDDPEMDGVLRSW
Section 5
(285) 285 290 300 310 320 330 340
Translation of BRW3-Consensus (direct 2) (271) QAQALVKEDKVVVEAIERRRAYVEANGIRPAMLSCDEAAV----------
Translation of Oxygenase sequence - P. Malto - DI6 (285) QAQALVKEDKVVVEAIERRRAYVEANGIRPAMLSCDEAAVRVSREIEKLEQLEAA-
Consensus (285) QAQALVKEDKVVVEAIERRRAYVEANGIRPAMLSCDEAAV

Alignment 3